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Protoplasmic streaming in Mucor

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INTRODUCTION

During the years 1903 and 1904 Alfred Schröter* carried out a series of experiments in the botanical institute at Leipzig under Pfeffer's direction on the subject entitled Über Protoplasmaströmung bei Mucorineen. The subject of Schröter's study was to investigate the effect of different external influences on protoplasmic streaming. Among the influencing factors he studied were light; temperature; injury, as the severing of a hypha or a sporangium; pressure, as upon a cover glass; influence of nutrient media; osmotic substances, especially of different concentration; and transpiration. In making the above study Schröter also desired to test the accuracy and application of the work of Ternetz† on the fungi he investigated.

Some of Schröter's experiments and conclusions, however, do not seem to be altogether above criticism, and I have found some of his results and statements to be incorrect, as the following pages will show.

Accordingly, during my recent study in Pfeffer's laboratory I began a reinvestigation of the various experiments of Schröter in order to ascertain to what extent they were defective. I have also extended the investigations of Schröter in some places and have given special attention to those experiments he performed which do not seem to be entirely conclusive.

* Schröter, Alfred. Flora **95**: 1–30. 1905.

† Ternetz, Charlotte. Jahrb. Wiss. Bot. **35**: 273–309. 1900.

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As to the historical account of the study of protoplasmic movements in the fungi it may be said that this has been given in sufficient detail by Schröter* and will only be mentioned here as reference makes necessary. Also Arthur† has made several references to brief accounts of protoplasmic movements in fungal hyphae. It will be seen by referring to the literature mentioned in this paper that the work of Woronin‡ appeared 31 years before the paper of Arthur. Since then a number of contributions on the subject of protoplasmic movements in the fungi have appeared, but the amount of study which this subject has received is very small in comparison with the attention that has been paid to such movements in the cells of other plants. And furthermore we find that protoplasmic movements in the fungi have been known for only about 45 years,‡ whereas in other plants such movement was observed 137 years ago, when Corti saw it, in 1774, as stated by Pfeffer.§

PLANT MATERIAL USED

In these investigations I have used *Mucor stolonifer*, *Mucor Mucedo*, and *Phycomyces nitens*. Of these three *Mucor stolonifer* and *M. Mucedo* were used in all my experiments. I found it inconvenient to use *Phycomyces nitens*, since as is well known the spores of this fungus seem to live for only a few months. I have been able at different times to keep some of the spores of *P. nitens* for as much as three months, but even then the majority of the spores with which I began to experiment refused to germinate. Therefore, in these experiments, while some spores germinated after a month or more, the uncertainty of their growth caused me not to use them so extensively as I did the other two forms above named. At all events it would be an interesting study and well worth investigating to ascertain, if possible, the reason for this short period of life that is shown by the spores of *P. nitens*. It is stated by Pfeffer‖ that the spores of most molds often live from one to three years. I have yet on hand the spores of *Mucor stoloni-*

* Schröter, Alfred. Flora **95**: 1. 1905.

† Arthur, J. C. Annals of Botany **2**: 491. 1897.

‡ Woronin, M., cited by Ternetz in Jahrb. Wiss. Bot. **35**: 274. 1900.

§ Pfeffer, W. Plant Physiology (Eng. Transl.) **3**: 289. 1905.

‖ Pfeffer, W. Plant Physiology **2**: 328. 1905.

fer and some of *M. Mucedo* that have kept for two years, and most of them still grow quite as well as the spores of these plants that are only a few days old. They have been preserved in a dry condition on bread, on which they grew in wide-mouthed bottles closed with cotton. This is also a very convenient way in which one may preserve the spores of those plants it is desired to grow—when first it is ascertained that the culture sealed in the bottle is a pure one. One may then by means of a sterilized needle or forceps easily remove a few spores to the desired culture media and in this way by a great saving of time and labor obtain pure cultures for study. Schröter* used *Phycomyces nitens* and *Mucor stolonifer* for his study; Arthur† used *Rhizopus nigricans;* de Vries,‡ *Phycomyces nitens;* Ternetz,§ *Ascophanus carneus;* and Woronin,‖ *Ascobolus pulcherrimus.* Of the three forms that I used, viz: *Mucor stolonifer, M. Mucedo,* and to some extent *Phycomyces nitens,* I found *M. Mucedo* also to be easily obtainable and as well adapted to the investigations of this paper as *M. stolonifer.* In addition, then, to my work on the other two forms my experiments with *Mucor Mucedo,* made to confirm or disprove Schröter's results, will also show whether or not the phenomena he and Ternetz describe are observable in still another fungus to the same extent as he mentions¶ for *M. stolonifer.*

APPARATUS AND METHOD

A description of the methods used in working out this paper can not all be described in one place, but the methods used in the various experiments can be materially shortened by describing and figuring at the outset a few of the principal pieces of apparatus used, to which reference may be made in the study where they were employed. For this purpose FIG. 1 is an illustration of the apparatus used. In some of the earlier experiments the apparatus shown in FIG. 1 was used in a somewhat more simplified form, but

* Schröter, loc. cit. 2.

† Arthur, loc. cit. 493.

‡ De Vries, H. Bot. Zeit. 44: 1–6. 1885.

§ Ternetz, loc. cit. 273–309.

‖ Ternetz, loc. cit. 273–309.

¶ Schröter, loc. cit. 10–29.

as the work progressed, some additions to it were found necessary for the sake of certainty of results and convenience. FIG. 1 was used especially for obtaining the hydrogen in a pure form. As here arranged, the gas generator *A* is to the left. The generator contained the purest zinc made. The sulphuric acid mixture used was composed of one part concentrated sulphuric acid diluted with nine parts of water,* and on its surface was poured a thick layer of liquid paraffin. The generator connects with the four-way stopcock *B* by means of which the stream of hydrogen may be sent to either of the U-tubes *C* or *C'*. Or the stopcock may be so arranged that the generator *A* is cut off from the rest of the apparatus and air drawn through *B'*. Of the U-tubes shown *C* and *C'* contain a concentrated solution of potassium hydrate, and in addition the arms of the U-tube are in each case nearly filled with pumice stone that has been saturated with the same solution. Hydrogen passed slowly through a tube arranged in this way will be freed from any hydrochloric acid, sulphur dioxid, or hydrogen sulphid that it may contain when prepared by the action of sulphuric acid on zinc.† The U-tubes *D* and *D'* contain silver nitrate for removing any traces of arsenic which may be present according to the equation:

$$6AgNO_3 + AsH_3 + 3H_2O = 6Ag + H_3AsO_3 + 6HNO_3.$$

The U-tubes *E* and *E'* contain potassium permanganate for the removal of organic substances and are connected with *F* and *F'* containing pyrogallol and concentrated potassium hydroxid for freeing the gas from any trace of oxygen.‡ It will be seen by the

* Andrews, F. M. Annals of Botany **19**: 523. 1905. "If, as sometimes happens, the sulphuric acid does not attack the zinc readily, so as to cause a rapid evolution of hydrogen, this may be brought about by the addition of a small quantity of platinum tetrachloride or copper sulphate to the sulphuric acid."

† Andrews, loc. cit. 523, where a similar but briefer method of washing hydrogen for another set of experiments is discussed.

‡ Hempel, W. Methods of gas analysis. Eng. Transl. 149. 1902. This gives the following formula for making an alkalin solution of pyrogallol which is a good and rapid absorbent for oxygen.

"5 grams pyrogallol dissolved in 15 ccm. of water,
120 grams potassium hydroxide dissolved in 80 ccm. of water."

"The absorptions do not take place well," says Hempel, "under 15° but since all my experiments, except those of temperature were above 15° the method could be used" and "A solution prepared as above stated gives off no carbon monoxide during the absorption."

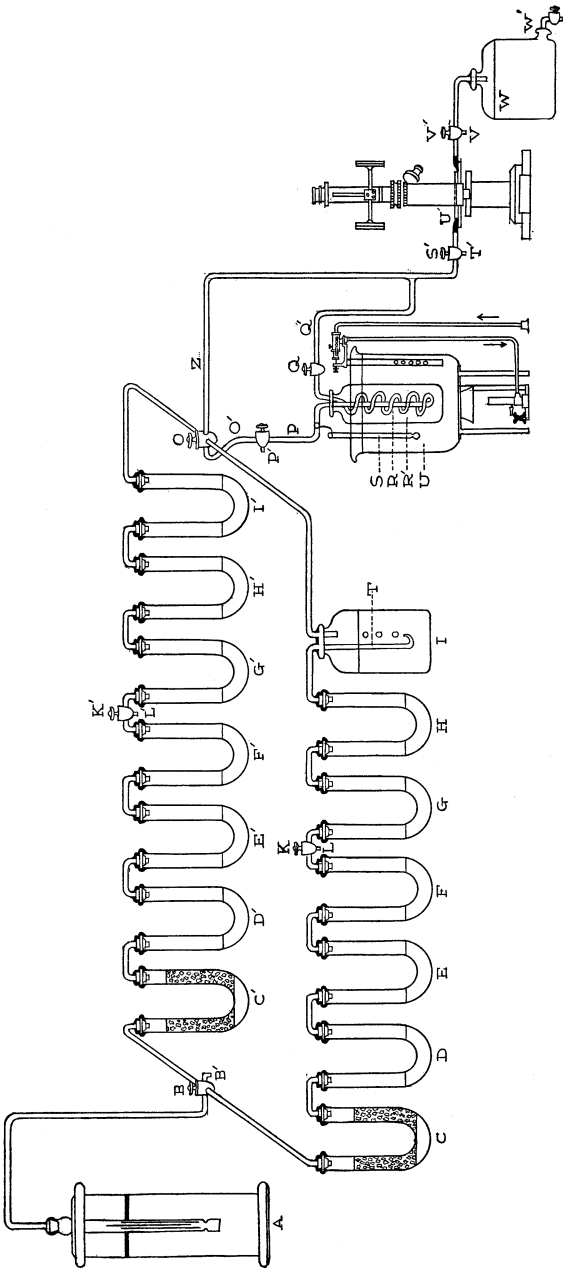


FIG. 1. Apparatus for controlling the temperature and purifying the gases used.

figure that a three-way stopcock is inserted between F and G and also one between the U-tubes F' and G'. This arrangement makes it possible to attach a tube at L or L', and without disconnecting or disturbing the apparatus to quickly draw off samples of the gas at these points and to ascertain if the chemicals in the various U-tubes are removing any objectionable substance from the gas and letting it pass through pure. The tests showed the hydrogen to be absolutely pure in the samples taken in this way. The tubes G and H and the bottle I contain pure water and pieces of wet filter paper to moisten the gas. Unless this is done the air which enters the gas chamber U' will soon cause the drops of water, or other medium in which the plants are growing, to evaporate.

The U-tubes G and H and the bottle I with the water they contain also act as a check valve to prevent a backward flow of any gas used. I used the bottle I instead of a third U-tube, so that a curved tube, T, might be inserted in it with a narrow opening of known size to assist in estimating the rapidity of flow of the gas.

It will be seen that C', D', E', and F' contain the same substances as C, D, E, and F, but experience has shown that it is the better plan to use two sets, and that it avoids a possible early deterioration of any of the chemicals which might occur before the experiments were completed, if only one set were used. The U-tubes G', H', and I' contain concentrated glycerin, and the arms of the tubes are loosely filled with pieces of filter paper saturated with the same solution to dry the air or gas drawn through these tubes.

From either of the two rows of U-tubes above mentioned the gas may be drawn through O' in two ways. It may go either through Z to the gas chamber U', containing the plants under investigation, or through the stopcock P, then through R to the gas chamber U'.

By the first way only gas of the temperature of the room is passed through U'. But by passing the gas through P and then R it may be warmed to any temperature in R by warming the water in R' as desired before sending it to the gas chamber. A more convenient way is to have the gas lamp X controlled by an accurate thermoregulator, or a still better method is to place

the vessel R', containing R, in a small well-regulated thermostat. Unless the generator A is very large it is rarely the case that a gas generated by it would be forced through the apparatus shown in FIG. 1. In order to make this possible an aspirator, W, is also attached and the flow controlled by a stopcock, W'. An aspirator may be graduated to estimate the outflow and gas drawn through, or the stopcock W' may be adjusted by means of a meter used on the outflow stopcock as shown by Detmer.*

If strong suction is not required a constant flow of any gas through the gas chamber U' may be obtained by the use of a floating siphon shown by FIG. 1*a* taken from the work of N. W. Lord.† The figure shown here is about $\frac{1}{6}$ the size of the apparatus I constructed for this work. It can not be used with the apparatus shown by FIG. 1 but can be employed when the gas has to pass through only a shallow liquid, or if gas only is to be drawn through. Its value lies in the constancy of the flow of gas it will cause, while other apparatus used for this purpose must be continually watched and regulated to insure accurate results. In some cases a suction pump, such as are attachable to water pipes, is an advantage to use. Wherever possible, and this was generally the case, all glass connections were made by having the tubes fused together in one continuous piece. (FIG. 1.) This made leakage impossible, which with hydrogen is very difficult to prevent under ordinary circumstances. The other connections, as for example with the generator, U-tubes, and the gas chamber, were made by means of rubber stoppers and sealing wax according to

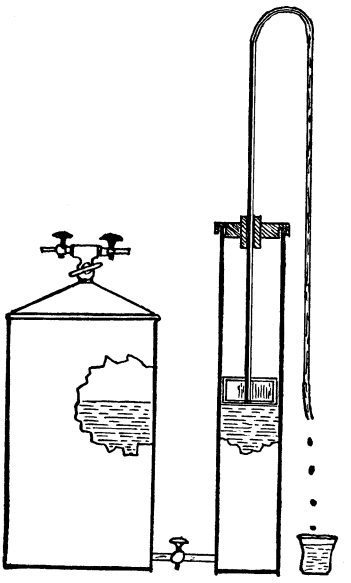


FIG. 1*a*. Floating siphon used to draw through a constant stream of gas.

* Detmer, W. Pflanzenphysiologische Praktikum 821. 1905.

† Lord, N. W. Notes on metallurgical analysis 181. 1903.

another efficient method I have used.* In some of my experiments, not shown in FIG. 1, I made connections between glass tubes in the following way, which were absolutely proof against leakage.

The method may be illustrated by the accompanying diagram, FIG. 2. Tubes of somewhat different but convenient sizes were used. The inner surface of *A* from *C* to *D* and the outer surface of *B* over the same distance were covered with paraffin and then these parts submerged in hydrofluoric acid. This etched the unparaffined surfaces of the tubes and allowed a more perfect and firmer grip of the sealing wax used in making the connection. The hydrofluoric acid was always carefully removed by washing and then drying the tubes before using the sealing wax. Or the tubes may be fastened gas-tight together by means of litharge and removed, when desired, by nitric acid as was done by Pfeffer.† One tube, *A*, had on its inner end, at *C*, a few layers of compact filter paper so as to make it fit *B* tightly. Very finely powdered sealing wax was then sifted in between *A* and *B* from *C* to *D*. Then, on gently heating the tubes from *C* to *D* in a flame the powdered sealing wax was carefully melted, and when it was cooled there was produced an absolutely tight connection. When it was desired to disconnect the tubes joined by sealing wax, the joint was gently heated to soften the wax, when the tubes were easily drawn apart. The joints made as above described were perfectly tight even when tested in water by a pressure of about one half an atmosphere. The U-tubes can be so arranged as to be totally under water as shown by Ewart.‡

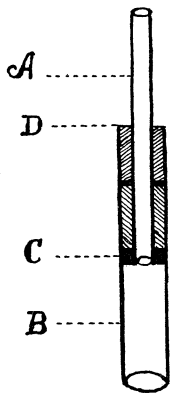


FIG. 2. Method of making glass tube connections by means of sealing wax.

A more simple and convenient way is to test the various joints by means of the large, short glass tube shown in FIG. 3. Each end of the large glass tube is closed by a split cork to hold the apparatus

* Andrews, loc. cit. 523.

† Pfeffer, W. Osmotische Untersuchungen 7, 12. 1877.

‡ Ewart, A. J. Protoplasmic streaming in plants 41. 1902.

on the tubes and make it water-tight. In case there should be vertical joints the same scheme as shown by FIG. 3 could again be used, if the lower end is closed by a split cork as before and the opening *D* closed by a rectangular piece of rubber or cork. Any leakage in a joint so surrounded with water would, of course, be easily detected, while the apparatus has the advantage of being quickly and easily changed from one connection to another.

In some cases metal gas chambers of the Engelmann type were used for the investigations, but in some others, where hydrogen especially was not used, the ring form of gas chamber was

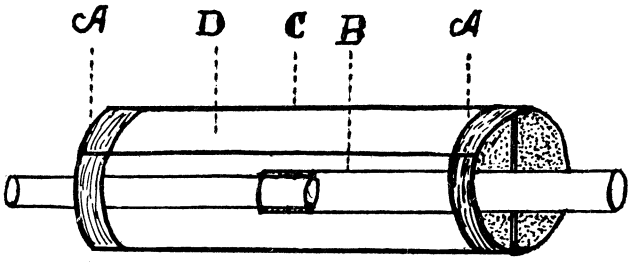


FIG. 3. Method of testing for leaks in glass tube connections under water. ×½.

employed. These were fastened to the slide with sealing wax or paraffin. A still better way is to use the glass ring form of gas chamber having a continuous glass base of its own.

In those cases where it was not necessary to pass gas of any kind over the specimens under investigation, simply glass rings were used and these were cemented to the slide by sealing wax or paraffin as above described. The cover glass to which the medium containing the specimens hung was made gas-tight by cementing it to the gas chamber, of whatever form used, by means of vaselin or a mixture composed of vaselin and wax as indicated by Pfeffer.* In some cases, as mentioned by Pfeffer, shellac of the proper thickness may be used to advantage especially in experiments requiring some considerable time. The gas chamber and apparatus were always thoroughly freed from any gas foreign to the investigation before beginning the experiments.

* Pfeffer, W. Plant Physiology 3: 239. 1905.

EXPERIMENTAL PART

The investigations were begun by a series of experiments to ascertain what culture media were best for the growth of these fungi. The specimens of *Mucor stolonifer* and *M. Mucedo* were grown in various nutrient media, which require a word of explanation here. Some of the spores of *Mucor Mucedo*, *M. stolonifer*, and *Phycomyces nitens* were observed to germinate very well in a 5 per cent solution of pure cane sugar. Other concentrations of the cane sugar solutions were used, but the 5 per cent solutions gave excellent results and under favorable conditions of temperature etc. a rapid growth resulted. In some experiments they grew quite well for a time in water, but this did not suffice for the conditions which the experiments of this study made necessary. Also a favorable medium was formed for the growth of the fungi in a tolerably concentrated solution of plum juice obtained in a suitable condition by filtering first through a thin layer of cheesecloth and then through filter paper. A solution made from horse manure caused a good growth, but it was inferior in this respect to sugar or plum solution, as was also a solution made by adding a little glycerin to pure water.

Another good medium for the growth of these fungi is bouillon made as follows:

50 grams lean beef,
100 c.c. water.

Let this stand from one to two hours at 50° C., then filter through cheesecloth. If less than 100 c.c. filter through add water to make that volume. Add 1 gram dry peptone and 0.5 gram of common salt, then boil, neutralize, clear, and filter.

In some experiments gelatin was used, made as follows:

50 grams lean beef,
gelatin 10 grams,
dry peptone 1 gram,
salt 0.5 gram.

Heat, neutralize, clear, and filter.

Agar-agar was also used. It was made by adding 15 grams of finely chopped agar-agar to the bouillon solution above mentioned. A concentrated solution of very saccharin grape juice was also a good medium for fungal growth.

Schröter also used several of the media I have just mentioned, but they differ in some respects as to the various ingredients and concentrations. I did not have good success in my experiments with citric acid on the fungi studied and abandoned it entirely after a few experiments. With the exception of the water and citric acid solutions I obtained good growths of large actively streaming fungi. The protoplasm sometimes moved as rapidly as from 2 to 4 mm. per minute, and it often maintained this movement to and from the apex for hours under the artificial conditions to be mentioned later, or as long as the observation lasted, which was frequently continued from morning till evening and recommenced the following day.

Some specimens of *Mucor stolonifer* and *M. Mucedo* were kept growing in a weak solution of grape juice and still others in a dilute solution of plum juice for five days. The streaming was apparent during all this time whenever the plants were placed under artificial conditions. In this culture medium the hyphae were small and the growth weak. They were grown in a hanging drop solution of the substances above referred to on a cover glass, and this was cemented to a glass ring cell by means of the wax mixture previously mentioned. The glass ring cell was cemented to the slide with soft paraffin. A better growth than in the last case, and also a specimen that lived longer, was produced by the spores of *Mucor Mucedo* in a 1 per cent solution of cane sugar. In this mixture most of the spores germinated, forming long hyphae. Many of these spores germinated very unevenly although all were mature. Some of them began to germinate only after two or three days or even longer, after others sown at the same time had produced hyphae of considerable length. The specimens were observed from time to time, and the movement of the protoplasm could be seen although not so rapid or in such volume as in those specimens grown in some of the nutrient media mentioned earlier in this paper. This protoplasmic movement was often evident almost as soon as germination was well begun and continued generally, when artificial conditions were introduced, for the entire life of the filaments. The growth shown by this experiment was not very rapid and the fungal filaments were small. Klebs* also

* Klebs, G. Bedingungen der Fortpflanzung bei einigen Algen und Pilze 506, 507. 1896.

has found that the nature of the nutrient medium was of great influence in causing a very large growth of fungal filaments where sugar was used. But this he found not to be the case for all sorts of sugar. He also found that the same applies to peptone solutions of certain concentrations. It has therefore been of importance in this study to select those culture media that will cause a rapid growth of fungal hyphae having large diameter so as to carry on the investigation to better advantage. The gelatin solution grew fungal hyphae of very large diameter and conspicuous streaming movements. The nutrient media, then, in some cases produced only small specimens unfavorable for study, while in other and good nutrient media large and favorable specimens for study were obtained. Such an investigation for suitable media is, as these experiments and the work of Klebs have shown, an extensive and valuable study in itself, but space does not allow a further consideration of it here.

In inoculating the various culture media above described a large steam-tight chest in Pfeffer's laboratory was used. The glass sides of this box were first carefully washed, and then live steam was rapidly passed into it from a steam boiler for one hour or so until well sterilized. All inoculations of the culture media with the desired spores could then be made within the glass box by proper care without the cultures becoming contaminated with bacteria or other forms. Unless this precaution was observed bacteria and other forms would frequently gain access to the cultures and interfere with the success of the experiment, or if it were continued for some time, frequently outgrow and destroy the forms under experimentation.

PROTOPLASMIC STREAMING IN MUCOR STOLONIFER AND M. MUCEDO

TRANSPIRATION

In these investigations a study was next made in order to ascertain how the protoplasm would behave when saturated air and the air of the room were alternated with each other. Accordingly the spores of *Mucor Mucedo* were placed in a hanging drop culture consisting of 5 per cent pure cane sugar and arranged in the glass ring cell as described above. The same was done in the case of spores of *Phycomyces nitens* and also those of *Mucor stoloni-*

fer, and in all cases large active growing hyphae were obtained. So far as could be ascertained from a great many cultures prepared in this way, all of the fungal spores just mentioned above seemed to grow about equally well. The temperatures were controlled by placing the cells containing the specimens in a constant temperature room in Pfeffer's Institute. A change of temperature, even though it be very slight, may sometimes cause an acceleration or retardation in protoplasmic movements. To prevent this the microscope and all apparatus concerned in such experiments were also put in the constant temperature room at the time the culture was started, in order to avoid a change of temperature, which would occur if the cell were placed on the colder surface of the stage of the microscope.

In such an investigation as this, where the temperature was to be considered, some experiments were carried on in the constant temperature room at 24° C. A more convenient way for the investigator is, in case the cover glass is properly attached, to make use of a warm chamber like that of Pfeffer* or that of Ewart.† Another rather convenient and exact way to control the temperature of the specimen for an indefinite period is by the use of the Molisch‡ freezing-box, in which the ice ordinarily employed is replaced by water of the desired temperature, which is siphoned in and out. The best and most exact method of controlling the temperature is to arrange an ordinary thermostat having an adjustable glass door. The microscope may be placed in the thermostat and operated as in the Molisch freezing-apparatus. The heat may be exactly regulated by means of a thermoregulator. In the study of the effect of temperature I did not attempt to ascertain what result the optimum temperature had on the hyphae of very different ages, if it has any, as is probable. Ewart§ has shown this to be the case in the cells of *Nitella* and *Chara*. At all events an investigation to ascertain this exactly in various fungi is much to be desired. The following two experiments will show the behavior of the protoplasm when saturated air and air

* Strasburger, E. Das botanische Practicum **3**: 22. 1887.

† Ewart, A. J. Protoplasmic streaming in plants 60. 1902.

‡ Molisch, H. Untersuchungen über das Erfrieren des Pflanzen 2–6. 1897.

§ Ewart, loc. cit. 63.

of the room are alternated and the specimens are grown under constant temperature. In the first experiment the spores were grown in a 5 per cent solution of cane sugar. The tables (I and II) will be self-explanatory with the exception of the first column, which indicates the number of times the saturated air and air of

TABLE I

EXPERIMENT WITH *Mucor stolonifer* GROWN IN 5 PER CENT SUGAR SOLUTION AT CONSTANT TEMPERATURE AND EXPOSED TO DRY AND MOIST AIR ALTERNATELY

Trials	Order of streaming	Direction of streaming	Time of streaming to tip	Time of streaming to base	Rate of streaming	State of vacuoles	Temperature
1	First	to tip	15 min.		rapid	Compressed	24° C.
2	Next	to base		20 min.	slow	Compressed	24° C.
3	Then	to tip	8 min.		slow, then faster	Compressed	24° C.
4	Then	to base		7 min.	slow	Compressed	24° C.
5	——	——	——	——	still for 3 min.	Compressed	24° C.
6	Next	to tip	9 min.		slow, then faster	Compressed	24° C.
7	Then	to base		4 min.	slow, then faster	Compressed	24° C.
8	Then	to tip	7 min.		slow, then fast, then stopped with jerk	Compressed	24° C.
9	Then	to base		5 min.	slow	Compressed	24° C.
10	Then	to tip	8 min.		slow, then fast	Compressed	24° C.
11	Then	to base		2½ min.	slow	Compressed	24° C.
12	——	——	——	——	still for 1 minute	Compressed	24° C.
13	Next	to tip	7 min.		slow	Compressed	24° C.
14	Then	to base		1½ min.	slow	Compressed	24° C.
15	Then	to tip	1 min.		slow	Compressed	24° C.
			Total 55 min.	Total 40 min.			

the room were alternated. The flow to the tip was caused when the dry air of the room was drawn through the gas chamber, and the flow from the tip when saturated air was admitted.

In this experiment the hyphae protruded from the sugar solution into the surrounding air of the gas chamber. It will be seen also

that while the protoplasm flowed 15 minutes longer toward the tip than to the base of the hyphae, the velocity of the apical stream

TABLE II

EXPERIMENT WITH *Mucor stolonifer* GROWN IN 10 PER CENT GELATIN AT CONSTANT TEMPERATURE AND EXPOSED TO DRY AND MOIST AIR ALTERNATELY

Trials	Order of streaming	Direction of streaming	Time of streaming to tip	Time of streaming to base	Rate of streaming	State of vacuoles	Temperature
1	First	to tip	10 min.		fast, then slow	compressed	24° C.
2	Next	to base		7 min.	fast, then slow	compressed	24° C.
3	Next	to tip	12 min.		slow	compressed	24° C.
4	Next	to base		3 min.	fast	compressed	24° C.
5	Next	to tip	5 min.		fast	compressed	24° C.
6	Next	to base		4 min.	fast	compressed	24° C.
7	Next	to tip	8 min.		slow	compressed	24° C.
8	Next	to base		2 min.	fast	compressed	24° C.
9	Next	to tip	11 min.		slow	compressed	24° C.
10	Next	to base		1 min.	slow, then stopped with jerk	compressed	24° C.
11	Next	to tip	4½ min.		slow	compressed	24° C.
12	Next	to base		15½ min.	slow	compressed	24° C.
13	Next	to tip	14 min.		slow	compressed	24° C.
14	Next	to base		3 min.	slow	compressed	24° C.
15	Next	to tip	19 min.		slow	compressed	24° C.
16	Next	to base		30¾ min.	slow	compressed	24° C.
17	Next	to tip	60 min.		slow	compressed	24° C.
18	Next	to base		70 min.	slow	compressed	24° C.
			Total 143½ min.	Total 136¼ min.			

was also much greater at first than the basal flow. The amount of protoplasm, therefore, carried in either direction over a given distance varied considerably during more than 1½ hours, during which time the observations of this experiment were continued.

A measurement of the distance traversed in the time given for the apical and basal stream would also substantiate this. In the second experiment the spores were grown in 10 per cent gelatin and under the same conditions as the previous experiment.

A glance at the figures in TABLE II will show that the total time of streaming to the tip in the experiment shown by TABLE II was also slightly greater than the time the protoplasm streamed to the base, by 7¼ minutes. An estimate of the time spent in streaming to the tip and base, taking into consideration the rapidity of flow of the protoplasm, shows that the difference as to time, as is above seen, is not very great in this experiment. The observations recorded in TABLE II were made from plants that were studied continuously for about five hours, and when the observations were discontinued at 8 P. M. the streaming of the protoplasm was still going on and could be seen again the next morning. The results of TABLES I and II in the two preceding experiments were also substantiated by numerous other such experiments not recorded here. Under natural conditions, however, if any difference could be detected in hyphae that were fully active in every respect, a slightly greater activity or flow toward the tip or young portions would be expected. To prove this would require uninterrupted observation from the time streaming first begins in the hyphae of these fungi. The streaming, as above mentioned, does not begin as soon as the spore has germinated, but only after the hyphae have attained some length. Good streaming hyphae were found in many cases after the spore had been sown about 18 hours, and nearly always 24 to 30 hours were sufficient at the optimum temperature to grow hyphae showing active protoplasmic streaming.

It is by no means always the case as Schröter* seems to think, that branching of the young hyphae is necessary for streaming to occur. It is true that no streaming of the protoplasm occurs when the filament is very young and entirely filled with granular protoplasm. As soon, however, as the filament has increased considerably in length and before any branches whatever have been formed, streaming is often plainly visible. Generally a much branched rapidly growing filament shows more rapid streaming than

* Schröter, loc. cit. 7, 8.

a slow-growing one or one without branches. Especially is this true in the case of an unbranched filament where the transpiration is influenced by artificial means. The sudden action of very dry air, for example, has a much more noticeable effect in producing streaming in the branched filament with large exposure surface, by the rapid withdrawal of water, than in the single unbranched filament with a much smaller exposed surface, in which streaming has just begun or in which it can for the first time be produced. The difference in the character of the cell walls of the branched and the unbranched filaments as to transpiration is unimportant in this connection. In unbranched filaments it is possible to cause streaming by artificial means before it would normally occur.

The two previous experiments show that a streaming of the protoplasm may be easily produced by artificial means. The artificial means in the instances just cited was transpiration. They also show that as transpiration is active or inactive, streaming is active or suppressed. The filaments grow when completely submerged, but the growth is not so rapid in wholly as in partly submerged fungal filaments.

In order to ascertain if streaming could occur in a saturated atmosphere, glass ring cells were made and the spores of *Phycomyces nitens* grown in the suspended 5 per cent solution of cane sugar. The filaments grew at the optimum temperature out of the solution into the completely saturated air of the closed cell, and when observed 48 hours later no streaming was present. In another cell similarly arranged the cover glass was slightly raised after the filaments had grown 48 hours. Streaming began in a few minutes and was very evident. This last arrangement allowed a slight interchange between the air of the artificial cell and the drier air of the room; and this small difference was sufficient to cause a rapid movement in the second experiment, and at the same time showed that while the change in humidity was slight, a little change in this respect may produce a considerable increase in transpiration and consequently a decided acceleration in protoplasmic streaming. The same experiments with the same results were carried out with *Mucor stolonifer* and *M. Mucedo*. The movements of the protoplasm shown by these experiments were therefore induced wholly by the transpiration occurring in that part

of the filaments protruding from the water, for in those spores that had germinated and whose filaments had not grown out of the hanging drop of sugar solution the streaming was absent. In every case of the many specimens examined streaming always began just as soon as a filament protruded from the solution into unsaturated air; and during the whole active life of the fungal filaments the velocity of protoplasmic streaming would be accelerated by artificial conditions, as the length of the filaments outside the drop became greater and the transpiring surface was increased. These specimens were kept growing in the glass cells for five days, at the end of which time the filaments had attained a great length, and in these rapid streaming was visible. Sporangia were also formed in both cultures.

In another experiment the spores of the fungi were grown under the cover glass on an ordinary slide in a 5 per cent solution of sugar or at times in water and the filaments allowed to grow from under the cover glass into the warm air of the constant temperature room or warm chamber. As before, so long as the filaments were completely submerged no streaming occurred, but as in the preceding experiment, as soon as the filaments emerged from the cover glass into the warm dry air an active streaming of the protoplasm commenced. In other experiments the fungal filaments were caused to grow from under the cover glass directly into a drop of water. When the water outside the cover glass was removed, streaming began immediately and was slow or rapid according as the amount of surface of the fungal filaments thus exposed to the dry air was small or large. The movements thus induced to the point where transpiration was occurring continued visible for a long time, or until practically all the movable part of the protoplasm was crowded as nearly as possible into the transpiring parts. When this occurred the vacuoles, which before were more or less elongated parallel to the long axis of the filaments, were now so crowded together and were so strongly compressed that their long axis was generally transverse to the filament. When water was again placed on any filament or filaments outside the cover glass from which it had been removed, a streaming movement immediately began away from the point at which such addition was made. This return movement continued, by this absorption, until equi-

librium was again practically established. Or if other parts were then exposed to the air the direction of motion was to them. In all cases excessive and prolonged transpiration in the ways indicated must be avoided to prevent a fatal termination. If this be done and care used, the number of times the protoplasm may be caused to stream to a transpiring part or away from what was a transpiring part, is unlimited. These experiments show, therefore, that transpiration causes streaming under the conditions here mentioned.

The dependence of protoplasmic movement on transpiration in the cases here referred to was further proved by growing the spores of the fungi mentioned, on the under side of a cover glass of an ordinary metal or glass gas chamber. Under these conditions, when saturated air was drawn through, the protoplasm did not stream in those filaments protruding into the saturated air. When, however, air that was nearly saturated was drawn through, slow streaming began. When very dry air was drawn through the gas chamber after the filaments had been in saturated air, streaming instantly began with great rapidity; and if this dry air was drawn through very long, the filaments were soon dried out and the exposed parts killed. The streaming finally stopped in those filaments in which it had been caused by dry air. When this occurred streaming was again produced by admitting air that is somewhat drier than that used to induce streaming before, if one does not delay too long before admitting the air. If after the use of the several degrees of dryness of dry air to induce streaming one readmits moist air in different degrees of humidity, streaming from the exposed parts of the filaments takes place for a time with a velocity in accordance with the humidity of the surrounding air. This movement continues till equilibrium is established, the time for which, however, will vary. The return streaming movement is slower and continues longer if the air is only partly made moist than if the air is saturated.

THE INFLUENCE OF HYDROGEN

In the filaments of *Mucor Mucedo* and *M. stolonifer*, grown as above described, hydrogen stopped the movement of the protoplasm in 20 minutes in moist air. Schröter gives about 5

minutes as the time required, but this seems a little too short a time in view of the average of 20 minutes which I found from experimentation with a large number of fresh plants. After streaming had been stopped by hydrogen it began again in one minute after fresh air had been readmitted. This experiment was repeated many times on the same filaments, always with the same effect. The streaming had in each case again become visible, but the effects had not entirely disappeared, as it responded less quickly after many trials.

Fresh specimens of *Mucor stolonifer* and *M. Mucedo* were not affected so soon by hydrogen passed through in moderately dry air as in saturated air. For example, it required pure hydrogen, as shown by many experiments, on the average 54 minutes to stop the streaming of the protoplasm in this plant in dry air. Also a somewhat longer time as compared to those in moist hydrogen was required for recovery. After streaming had been stopped in dry hydrogen, 5 minutes instead of 1 minute were required for recommencement of streaming after fresh air had been drawn through.

The protoplasmic streaming of fresh specimens of *Mucor stolonifer* and *M. Mucedo* was again stopped by a stream of saturated hydrogen in 20 minutes. When this experiment was performed the streaming was at first toward the base. Then, when dry hydrogen was passed over the specimen, the streaming began to the tip and continued for one-half minute. Again, moist hydrogen was admitted and streaming began to the base, stopping in 15 seconds. Then dry hydrogen was passed through and streaming began to the tip, ceasing in 10 seconds. This experiment was repeated ten times with the dry and moist currents of hydrogen, alternating them each time, always with the same result that streaming was reinduced each time. The moist hydrogen was easily obtained by causing it to pass through *G*, *H*, and *I* before entering *U'*, FIG. 1.

In another series of trials like the preceding, but on another specimen, the length of time the protoplasm streamed from the moist or to the dry hydrogen, respectively, is shown by the following experiment, TABLE III:

TABLE III

Trials	Streamed to the dry hydrogen	Still	Streamed from the moist hydrogen
1	2 minutes fast		1 minute fast
2	½ minute fast		5 seconds slow
3	23 seconds slow		3 seconds slow
4	1 minute very fast		10 seconds slow
5		5 seconds still, starts with jerk	
6	5 seconds very fast		2 minutes very slow
7		3 seconds still, starts with jerk	
8	20 seconds fast		14 seconds slow
9	12 seconds fast		9 seconds slow
10	5 seconds fast		3 seconds slow
Totals	275 seconds		224 seconds

It will be seen from the above figures that the time of flow in either direction varied greatly. The greatest amount of time, as well as the greatest velocity of streaming, was to the dry hydrogen. In only the first trial with moist hydrogen did the streaming appear fast. The totals of time above given and also the velocities observed are much less, as a rule, than where dry and moist air were used. The specimens used in the above experiment were replaced in fresh air and put away for 24 hours, at the end of which time they were living and streamed as actively as before. The air used in the experiment just mentioned was made perfectly dry by passing it over pure glycerin (FIG. 1). Other experiments, however, were performed in which weaker solutions of glycerin were used. It is sufficient to mention here one instance in which 30 per cent glycerin was used. The streaming to the dry hydrogen in this case was much slower, as expected, and continued for 7 minutes, which is a much longer time than the total of the same number of trials as in the previous experiment.

As has been shown above, transpiration may be practically suppressed and streaming stopped or prevented by a saturated air in 20 minutes. When saturated hydrogen is passed through under precisely the same conditions, the streaming stops in less time than in saturated air alone. This was shown by causing saturated air to be drawn over the filaments of *Mucor stolonifer* and *M. Mucedo*, which stopped streaming in 45 minutes by preventing transpiration. When, however, saturated pure hydrogen

was drawn over the specimens the streaming of the protoplasm stopped in 20 minutes. This is the average time of many experiments of this kind with both saturated air and saturated hydrogen. As there was a difference shown by these separately, it was thought that a difference could be proved when they were used successively on the same specimen. Accordingly, pure saturated hydrogen was used again on a fresh specimen and the streaming stopped in about 20 minutes as before. Just as soon as streaming ceased, saturated air was drawn through and immediately a slow streaming of the protoplasm began and continued for 8 minutes. This and other experiments indicate that when the streaming of the protoplasm is controlled or influenced by transpiration this may be partly caused, as in this experiment, by physiological action. This agrees with Schröter's opinion concerning transpiration in these fungi. When spores were sown in small drops of the solution of the different liquid nutrient media and a rapid growth occurred so that numerous long and rapidly transpiring filaments projected into only moderately dry air, the streaming of the protoplasm produced by transpiration continued often for several hours. This finally resulted, as Schröter states, in a concentration of the various liquid nutrient media to an extent that streaming to the transpiring or formerly transpiring tips finally ceased. If the small drops of liquid nutrient media be again diluted, streaming in the former direction will occur; and this experiment may be repeated a good many times with the same specimen, and always with the same result if due care is observed.

In these experiments on transpiration the air or hydrogen that was drawn over the specimens was warmed (in *R*, FIG. 1) before coming in contact with the plants under investigation. In all cases when hydrogen was employed in the experiment, suitable bacteria were used in order to ascertain that no oxygen was present to interfere with the results.

A series of experiments was next tried to show the effect on transpiration of different strengths of glycerin, also glycerin in combination with other reagents. The glycerin mixtures did not in any case come in direct contact with the nutrient media in which the fungi experimented with grew and also did not touch the exposed fungal filament when these projected from the culture media. The

spores of the fungi were grown in hanging drop cultures over the glycerin solutions in the ordinary glass ring gas chambers. When good growing and streaming specimens were obtained they were placed, in the first of these experiments, over a 50 per cent solution of glycerin for 15 hours. This was much longer than it had been intended to allow the experiment to continue, and at the end of that time, as was expected, the specimens were all killed by being completely dried up and collapsed beyond easy recognition. This takes place in the filaments of the fungi here studied in a much shorter time, as mentioned, than 15 hours; for when the experiment was repeated, with 50 per cent glycerin, it was found that the fungi were desiccated to a point beyond vital recovery in 47 minutes.

In a third experiment of this strength of glycerin it was found that when a specimen of each of *Mucor stolonifer* and *M. Mucedo*, which were growing in a saturated air, were changed to a cell over a 50 per cent solution of glycerin, all streaming of the protoplasm stopped in 15 minutes. When the specimen was first placed over the glycerin solution the streaming, which to begin with was absent, began almost instantly and continued for a few minutes with great speed to the exposed portions of the filaments from which the water was being rapidly removed by vigorous transpiration. As the air became drier the streaming which at first was so rapid became in a short time slower and slower, as the water was removed from the filaments, and in a little longer time, as stated, stopped in 15 minutes. When the specimen was then changed back over a cell containing pure water or one through which saturated air was passed, the streaming recommenced in 5 minutes on the average. This recovery of the streaming under these restored conditions was at first slow but in a few minutes increased in velocity from the tips or exposed portions which had formerly transpired rapidly. The increase in velocity of streaming continued till the filaments were again about turgid or equilibrium was established, when it finally ceased, as it did when they were in the saturated air before being placed over the glycerin. This experiment can be repeated many times with the same active specimen if care is taken not to allow desiccation to progress too far. When the transpiration has been excessive for some time, the filaments are often

more or less collapsed or their diameter somewhat reduced after the loss of a considerable quantity of water. Even when all streaming has ceased and collapse of the filaments has then occurred to a quite noticeable degree, they may recover their normal dimensions and streaming recommence if they are surrounded with moist air from which the water is reabsorbed by the filament. In one experiment, when this collapse occurred in 22 minutes in air dried by 50 per cent glycerin, recovery in saturated air took place in 52 minutes and streaming reoccurred. Collapse and cessation of streaming can easily be produced over 50 per cent glycerin solution in considerably less time than it is possible to restore the filaments fully to the normal condition.

As was to be expected, when the solutions of glycerin employed were made weaker, the effect on streaming as the result of weaker transpiration was less pronounced. For example, when *Mucor stolonifer* and *M. Mucedo* were placed over a 20 per cent solution of glycerin the streaming stopped in one hour. During all this time the streaming was, as in the 50 per cent glycerin, to the tips exposed to the air that was being dried by the glycerin. The streaming was not so rapid as over the 50 per cent glycerin. After replacing the specimen in saturated air streaming recommenced and regained its normal velocity much sooner than when left an equal time over 50 per cent glycerin. Frequently all that is necessary to produce streaming in such fungal filaments which apparently are inactive, is to cause transpiration by drying the air around them with glycerin and as a rule only slightly. The effects of still weaker solutions of glycerin will be seen by the following experiments. When the fungi were placed over a 10 per cent solution of glycerin the transpiration was so diminished that streaming continued for 3 hours to the portions exposed. When they were placed over a 7½ per cent solution slow streaming was to be seen to the transpiring parts for 5 hours. When they were placed over a 5 per cent solution of glycerin the streaming was still visible 8 hours after the experiment was started, and the filaments were not perceptibly dried up. The transpiration was very slow and streaming was always to the exposed parts.

In several of the experiments, where the spores were grown under the cover glass, bubbles of air of different sizes were also

present. In those cases where a good many spores were grown under the same cover glass some of the filaments or their branches just entered or passed entirely through some of the air bubbles. In these, however, the air was saturated and no streaming occurred. Even when they had just grown into the bubble no streaming was seen. When the position of a saturated bubble under the cover glass was shifted so that it was placed over a filament or a part of a filament that had previously been entirely submerged, no streaming of the protoplasm began. This shifting can be easily done by very slight pressure on the cover glass when it is nearly swimming in the solution which submerges the filaments. If this pressure is properly applied, a bubble which is a little distance from a filament can be caused to flow over it. At the same time, since the cover glass does not rest directly on the filaments, the pressure is not sufficient in this case to cause any injury, as the experiments showed. If, however, a dry air bubble is gently brought under the cover glass directly in contact with the filaments by means of a glass capillary tube, streaming will be induced immediately and will continue for a time. A convenient way of growing the filaments so as to prevent them from from being affected by pressure in moving a bubble around under the cover glass is to make a cell of a layer of filter paper and fill it with the solution and a few air bubbles. For the use of the capillary tube here mentioned the filter paper cell should be cut away on two sides to freely admit the tube. At first I forced air bubbles under the cover glass through a very fine capillary tube by means of an automobile pump. By the use of the automobile pump directly connected it was difficult to regulate the flow and constancy of the air bubbles. To overcome this difficulty the air was forced directly into an ordinary autoclave, *A*, as an air receiver, by the pump *B* and from this was conducted through the capillary glass tube to the specimen. A mercury manometer, *C*, was connected with the apparatus to show the pressure more accurately than the autoclave gauge would do. From $\frac{1}{7}$ to $\frac{1}{2}$ of an atmosphere was necessary to force the air quickly through the capillary glass tube *D*, according to the size used (FIG. 2*a*). By care, with this method a number of bubbles or a stream of them may be forced in, and their number and size con-

trolled by the dimensions of the glass capillary tube, which is often of importance. Small bubbles may in this way be brought to only a part of a long filament and the effect studied. A stream of small bubbles generally quickly ran together when they touched a filament near one another. It was possible by this means to expose for a time to dry air, only one side of a filament that had previously been completely submerged. The streaming, which occurred due to the bubble of dry air forced in through the glass capillary, was slight if only a small part of the filament was touched or covered. If a number of bubbles were let in, so that a larger part of the filament's surface was exposed to dry air, the streaming was somewhat faster, and it continued to increase according to the amount of surface thus suddenly exposed to the dry air.

In these experiments the streaming was never so fast and did not continue so long as in the experiments where the fungal hyphae were exposed to moist and then dry air in a gas chamber. The streaming was always to the part exposed to the dry air where transpiration was taking place. When the filament was resubmerged, streaming occurred for a time away from the part that had been exposed to dry air, but it was never so rapid as it was to the tip when this part was in dry air. These experiments are also a confirmation of all those investigations in which it was proved that in a saturated air in a gas chamber streaming which had begun ceased, and when dry air was let in began again. What is practically the same is that streaming may be induced by forcing warm dry air by means of a glass capillary tube gently among the fungal filaments in a hanging drop, if it be continued long enough and if the stream of bubbles be rapid. The streaming was in the latter experiment slower than in the preceding experiment. Some of the bubbles were caught in the mesh of fungal filaments and held; and in these cases where they touched and remained in contact with the filaments streaming was induced to that point, but after a few seconds or minutes it ceased.

The method of using a capillary glass tube was not at first so easily manipulated, and many trials and the loss of a good many specimens resulted before it was successful to my entire satisfaction.

Some other specimens for these experiments on transpiration

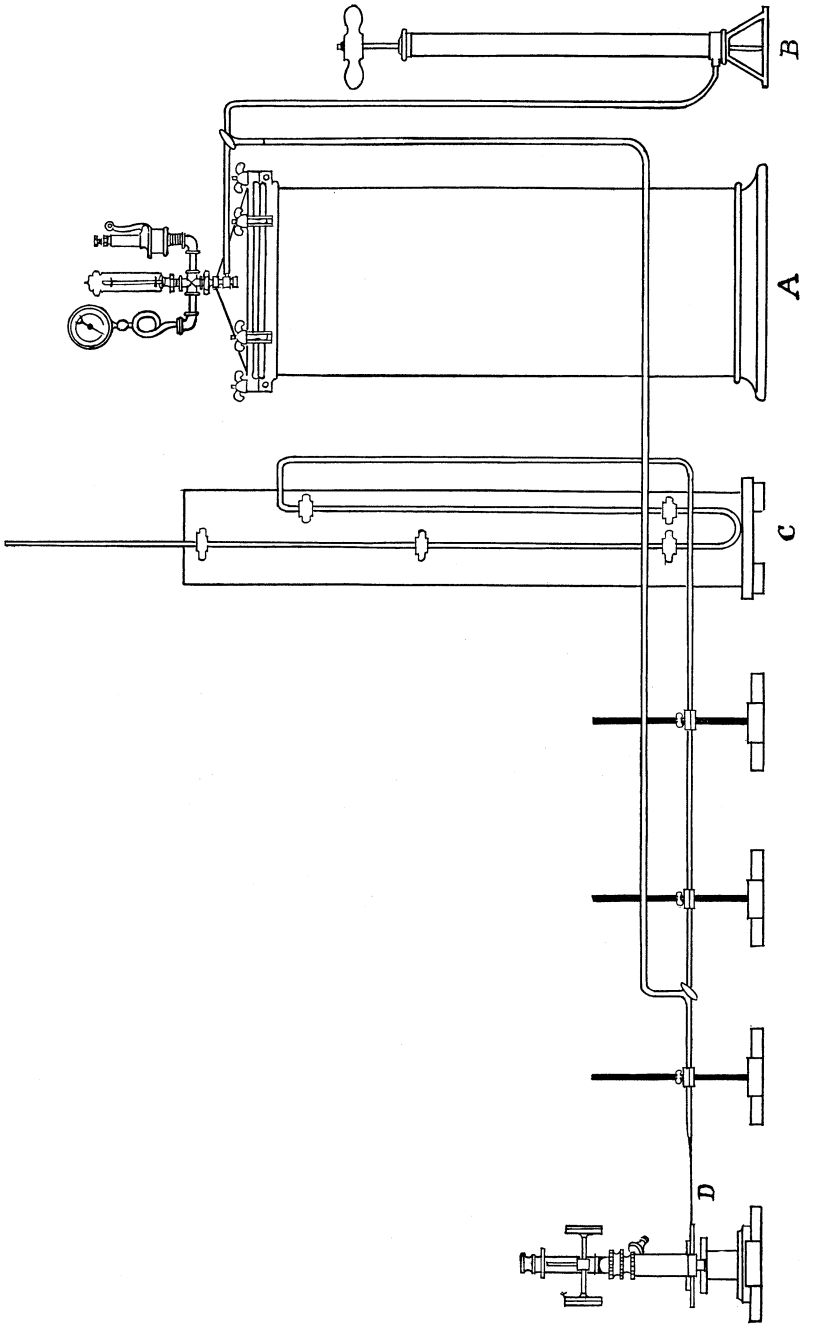


FIG. 2*a*. Apparatus for applying bubbles to the specimens under the cover glass.

were grown in a 10 per cent solution of gelatin. Under favorable conditions this medium produced large actively streaming specimens. The various fungi behaved in this 10 per cent solution of gelatin in the same way when subjected to artificial conditions to produce or suppress transpiration, as they did in the solution of cane sugar. For example, when the streaming of *Mucor Mucedo* and *M. stolonifer* had been stopped in a saturated air and the plant was then placed over a 20 per cent solution of glycerin, the streaming began and moved rapidly to the exposed tips at first but gradually became slower and then stopped in 50 minutes. This was the average time required under such conditions, which will be seen to be a somewhat shorter time than when the filaments grown in a cane sugar solution were placed over a 20 per cent solution of glycerin. It was probably partly due to the lack of easily available water in the glycerin medium so that transpiration could not continue so long. When the spores were grown in a gas chamber and saturated air drawn through, the streaming ceased, but began again when dry air was passed through the cell. The velocity of streaming was dependent on the dryness of the air as prepared in the apparatus shown in FIG. 1.

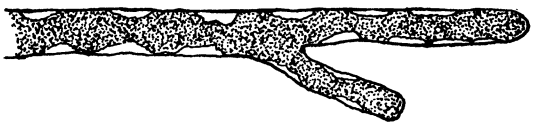


FIG. 4. Part of a filament of *Mucor stolonifer* partially collapsed by excessive transpiration. × 400.

In all the experiments, when transpiration had proceeded too far the protoplasm was drawn wholly or partly away from the cell wall and gave the appearance shown by FIG. 4. In such cases as in FIG. 4, if moist air was readmitted, the protoplasm in a short time resumed its natural position and streaming recommenced.

EXPERIMENTS WITH ETHYL ETHER

When the same specimen as used above, whose protoplasm stopped in 50 minutes over 20 per cent glycerin, was laid over a cell containing 20 per cent glycerin to which a ¼ per cent solution of ether had been added, streaming began again, more

slowly at first; then placed over the 20 per cent glycerin alone its streaming continued slowly for 15 minutes to the tip. When this specimen was again placed over pure water streaming began very rapidly from the tip and continued so for 10 minutes, but again it moved to the tip when the air was slightly dried. The ¼ per cent solution of ethyl ether used in this experiment was still further weakened to ⅛ per cent when it was added to the 20 per cent glycerin. The same experiment was carried out over a full ¼ per cent ether. As above mentioned a temporary awakening of the protoplasmic activity occurred. Schröter* states that a ¼ per cent solution of ether in a glycerin solution will stop streaming, but he does not state the concentration of the glycerin or the time required. This I also found to be true as above stated, but he has evidently overlooked the fact that a very weak solution of ethyl ether in dilute glycerin may cause a temporary recommencement of streaming if the ether is immediately applied. In either case it shows that transpiration is not purely mechanical but that certain physiological factors enter into the process. The above experiment is not easy to perform, owing to the liability of the protoplasm to lose too much water before the ether may be properly caused to act upon it. A reawakening of protoplasmic activity resulting in earlier or increased growth has been shown for other plants by Johannsen.† While this is true of small amounts of ether, by increasing the dose to a certain concentration not only a mere awakening of activity was caused but a more rapid growth.‡ In these experiments with a weak solution of ether on the fungal filaments a great many groups of the fungi were grown on a glass plate which fitted a large glass cell tightly. An apparatus like FIG. 5 is convenient to use to quickly draw into a cell a solution of glycerin and ether. By opening the stopcock *A* and applying suction at *C* the 20 per cent glycerin is drawn out, and then by turning the three-way stopcock *A* again and opening *B* the desired glycerin and ether mixture in *D* is drawn into *E* under the specimens at *F*.

Strong solutions of ether in glycerin caused an unfavorable

* Schröter, loc. cit. 29.

† Johannsen, W. Das Aether—Verfahren beim Frühtreiben 61. 1906.

‡ Johannsen, loc. cit. 61.

effect. For example, when the specimens were placed over a 20 per cent solution of glycerin plus a 1 per cent of ethyl ether, streaming did not recommence as in the preceding experiments. When a new and actively streaming specimen was placed over the mixture, streaming ceased in 24 minutes. The protoplasm streamed

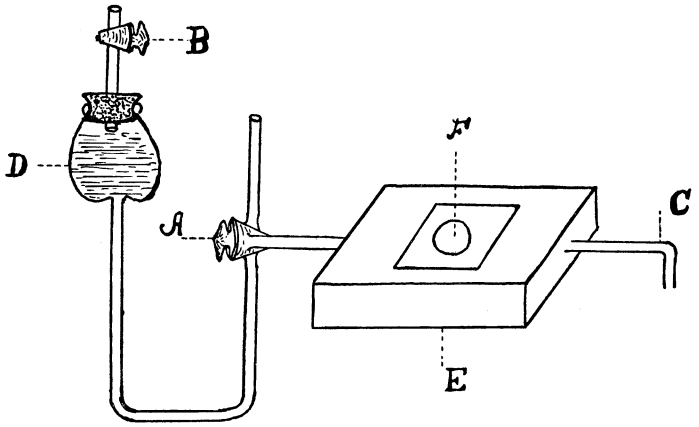


FIG. 5. Apparatus for drawing a solution into the gas chamber.

fast at first over this solution but became gradually slower till it stopped. The specimens were not killed, for when they were placed over water or saturated air drawn through the cell, streaming began again. The movement was at first to the base then to the tip of the filaments again when the air was only slightly dried. The time required for a recommencement of streaming under these conditions varied according to the state of desiccation; but on the average, where streaming had just ceased, it began again in moist air in about 3 minutes. The movement was at first from the tip, where absorption was occurring, and then to the tip again when the air of the room, which was slightly drier than that of the cell, was admitted.

EFFECT OF OSMOSIS

For the study of the effect of osmosis on protoplasmic streaming in fungal filaments the spores of *Mucor Mucedo* and *M. stolonifer* were grown in water at a constant temperature on a glass slide. The cover glass was supported by bits of a cover glass so as not to rest on the spores or filaments and to admit a capillary tube·

A small capillary tube which had been filled with a 5 per cent solution of cane sugar was then carefully inserted under the cover glass and so placed that its open end was near one of the fungal filaments. In 10 minutes the sugar solution had diffused out in the water and come in contact with the filaments, and the protoplasm began to stream very fast to the point by the open end of the capillary tube. Sometimes the induced streaming was slow and then suddenly became more rapid. The capillary tube offered a convenient method of enabling one to bring the sugar solution to any desired part of a filament and thus study the behavior at certain places. No perceptible difference could be detected in this way between the base and apex of filaments capable of active streaming. At whatever part of an active filament the capillary tube containing the 5 per cent solution of cane sugar was applied streaming to that point was always induced and in a very short time. As was to be expected, weaker solutions of the cane sugar required more time and produced slower streaming while more concentrated ones caused more rapid streaming in less time. This method of using the glass capillary tube is not quite so simple as it may seem, as certain mechanical difficulties are experienced, especially when it was used with objectives of sufficient power to see the streaming distinctly.

A somewhat similar experiment in principle is one in which streaming almost immediately began in the filaments when a drop of sugar solution was brought to the edge of the cover glass. Here, however, the movement stopped sooner than when the solution was applied at one place by means of a capillary tube, since it diffused all around the filaments and the water was more quickly and uniformly withdrawn.

I can confirm the experiment of Schröter* in which he found that a sugar solution added to one side of the cover glass caused streaming in that direction, and I found that it continued from 2 to 17½ minutes according to concentration and the condition of the filaments. This is the time for both *Mucor Mucedo* and *M. stolonifer*. Also, I have found that the excessive loss of water by a sugar solution results in a great decrease in the size of the

* Schröter, loc. cit. 21, 22.

filaments and in some instances a breaking of them, allowing the contents more or less completely to escape. By adding the sugar solution first to one end and then to the other, of a filament or a mass of them, and washing away the sugar after each application before reapplying it to the opposite end, the protoplasm may be made to stream to the sugar at each new application. If the sugar solution is applied to one side, movement occurs in that direction and then stops as above mentioned. If then the sugar solution is washed away with pure water, the part of the filament toward which streaming occurs will take up water, and streaming will take place away from this part for a time, or until equilibrium has been established. After stoppage in this way, the addition of even a weak solution of sugar at the part toward which streaming was moving causes it to recommence in this direction.

The following experiment will show the effect of sugar and give an idea of the number of times streaming may be induced and the duration of each period of streaming. To do this a glass slide was supported at each end on a glass block in a petri dish, the latter to hold the liquids that ran from the slide. The spores of *Mucor Mucedo* and *M. stolonifer* were grown in a very small drop of gelatin. Two wet strips of filter paper were so arranged that the edge of each just touched the edge of the cover glass and their ends hung down some distance into the petri dish. On one of the strips of filter paper was placed a lump of cane sugar, which was renewed as fast as necessary. As soon as the sugar was moistened and the solution passed under the cover glass, streaming to the sugar began at once. When streaming ceased, drops of water were added to the other strip of filter paper, which washed away the sugar solution, causing streaming in the reverse direction. The velocity of streaming in this experiment, especially toward the sugar solution formed in this case by the dissolving lump, varied. This was due to the unequal solubility of the sugar under the conditions here presented, which caused the solution to vary in concentration. At other times the sugar solution did not diffuse or pass under the cover glass with equal rapidity, owing to the fact that it was impossible more than approximately to control the amount of water supplied to or removed from the specimen. The number of times this experiment was performed and the

duration of streaming in each case is shown by the following TABLE IV:

TABLE IV

Trials	Streamed to sugar	Stream reversed to water when added	Still	State of vacuoles	Temperature
1	7 min.	——		compressed	24° C.
2	——	11½ min.		compressed	24° C.
3	6 min.	——		compressed	24° C.
4	——	10 min.		compressed	24° C.
5	10½ min.	——		compressed	24° C.
6	——	3 min.		compressed	24° C.
7	17½ min.	——		compressed	24° C.
8	——	4 min.		compressed	24° C.
9	12½ min.	——		compressed	24° C.
10	——	2 min.		compressed	24° C.
11	6 min.	——		compressed	24° C.
12	——	——	still 4 sec.; starts with jerk to sugar solution	compressed	24° C.
13	8 min.	——		compressed	24° C.
14	——	14 min. very slowly		compressed	24° C.
15	9½ min.	——		compressed	24° C.
16	——	12 min.		compressed	24° C.
17	7 min.	——		compressed	24° C.
18	——	16 min. slow		compressed	24° C.
19	5 min.	——		compressed	24° C.
20	——	——	still 8 min.; starts with jerk to water	compressed	24° C.
21	2½ min.	——		compressed	24° C.
22	——	6½ min.		compressed	24° C.
23	1 min.	——		compressed	24° C.
24	——	20 min.		compressed	24° C.
	92½ min.	99 min.			

The experiment, as will be noted, was continued about three hours. The streaming was always faster to the sugar than to the water. Even an incomplete return of the protoplasm to the water before cessation of movement required a longer time than a complete transfer of all movable contents to the sugar, as is shown by the total time required for streaming in either direction. As will be seen, the total time the protoplasm was observed to move to the sugar during about 3 hours of constant observation was 92½ minutes, while the movement to the water for all the different times amounted to 99 minutes, or a difference of 6½ minutes. To this, as stated above, must be taken into consideration that the streaming to the sugar solution was much faster than to the water.

On two occasions, as will be observed by referring to TABLE IV, the streaming stopped once for 4 and at another time for 8 seconds to the sugar and to the water, respectively, and in starting did so with a jerk. After the 3 hours of experimentation with the fungal filaments in the above mentioned manner the specimen was put away for 24 hours at the optimum temperature to see if this treatment had had any detrimental effect. It was again observed at the end of this time and found to be in a perfect condition, and it again responded to the reagents which produced streaming as readily as when the experiment was at first begun. This experiment is similar to the one performed by Schröter* with a sugar solution and water, which I have also repeated and can confirm.

The streaming may also be caused to recommence when it has been stopped by cold. Specimens of *Mucor Mucedo*, *M. stolonifer*, and *Phycomyces nitens* grown in 10 per cent gelatin ceased to show streaming when suddenly transferred from a temperature of 24° to 9° C. When a 5 per cent solution of cane sugar was added, streaming began again in 15 seconds, but as is to be expected, not so rapidly as in specimens that were kept at optimum temperatures. The same specimens were then subjected to a temperature of 5° C. after washing out the sugar with water; and in this case too streaming was induced by a 5 per cent solution of cane sugar, but a longer time was required. The streaming was feeble and was not seen until one minute after the sugar was applied. The direction of motion was to the sugar solution, which was applied first to one end and then the other with previous washing with water before each new application. It is not necessary to use as high a concentration of sugar (10 per cent) as mentioned by Schröter.† A higher concentration of sugar than 5 per cent will start streaming in less time and more rapidly than mentioned above. No matter what concentration of sugar solution was used, the protoplasm did not flow as freely or as rapidly as when the plants were growing under the most favorable circumstances. The most favorable results are obtained if the sugar solution is applied immediately after streaming has been stopped by cold.

* Schröter, loc. cit. 21, 22.

† Schröter, loc. cit. 21.

It has been shown that chloroform may stop streaming if used in a strong solution, but if used in a very dilute solution it may accelerate streaming or even cause it to begin.* The same is true of ether as regards the fungi used for this investigation. The solution of ether used to cause streaming to begin must be very dilute and the protoplasm be in a condition to stream. I have not experimented with chloroform on the fungi mentioned in this paper.

Specimens of *Mucor Mucedo* and *M. stolonifer* were grown in 10 per cent gelatin, and then a solution of ⅛ per cent ethyl ether was added. The streaming ceased in a few seconds after the ether was applied but began again slowly in 15 minutes. This same experiment was tried on other specimens of these same fungi with the same results. A quantity of the ⅛ per cent ethyl ether solution was also placed in the bottom of the glass cell below the specimens. As soon as it was found that streaming ceased for some time after the direct application of ⅛ per cent ether, a 4 per cent solution of cane sugar was added immediately to the specimens whose streaming had just ceased due to the ether. Streaming began again in three seconds, and all movement was to the parts of the filaments with which the sugar solution came in contact. When the sugar solution was washed away and water added, the streaming began but in the reverse direction.

Another series of the same fungi were grown as in the preceding experiment, but this time a ¼ per cent solution of ethyl ether was added directly to the specimens growing in 10 per cent gelatin. The protoplasm stopped streaming almost as soon as the ether was applied. The specimens were not killed by the addition of ether of the above strength, for when the ether was replaced with water the streaming recommenced and after a time regained its normal velocity. If a 4 per cent solution of cane sugar was applied immediately to the specimens that had just ceased to stream due to the ¼ per cent ether, streaming began in from 10 to 15

* Hauptfleisch, P. Untersuchungen über die Strömung des Protoplasmas in behäuteten Zellen. Jahrb. Wiss. Bot. **24**: 220. 1892; Ewart, A. J. Protoplasmic streaming in plants 87. 1902; Pfeffer, W. Plant Physiology **3**: 319. 1905; Josing, E. Jahrb. Wiss. Bot. **36**: 210. 1901.

† Ewart, loc. cit. 86; Josing, loc. cit. 210.

seconds. The specimens for these experiments were grown at the optimum temperature and treated in exactly the same way except that in the last case the strength of the ether was greater. The amount of time, however, required for the resumption of streaming in the last case was much increased. If the cane sugar was weakened, as was done in this case by using only a 3 per cent solution, the streaming began again in about 25 seconds. When the ether was washed away from the still protoplasm with water, streaming began as before.

When specimens prepared as the above were covered with a 2 per cent solution of cane sugar, streaming recommenced in about one minute on the average but was less active than in the stronger solution of sugar. A 5 per cent solution of sugar caused streaming to commence in 30 seconds, and a 10 per cent solution of cane sugar caused a recommencement of streaming under the above conditions, almost instantly and very actively in all cases, to the sugar.

When the specimens are placed in a ½ per cent solution of ether, streaming stops immediately, but it can be caused to recommence by adding a sugar solution if this be applied at once. A 5 per cent solution of sugar caused streaming to commence in 30 seconds. The time, however, will vary according to the strength of the solution, as shown above, and the length of time the ether has acted. In respect to the water solutions (½ per cent ether) above mentioned I can confirm the experiments of Schröter.*

A saturated solution of ethyl ether was added directly to the filaments, and streaming ceased instantly. No acceleration of the streaming occurred before it stopped. When the ether was washed away at once with water streaming did not recommence.

A 1/20 per cent solution of ethyl ether directly applied to the fungal filaments causes streaming to begin in them when they are in a condition for such activity. This movement continued for about the usual time, and when finally the dilute ether was washed away with water the streaming continued. This was true of any filaments whether they were branched or not.

A convenient way to apply the cane sugar in any case where

* Schröter, loc. cit. 21.

an exact per cent is not required is to place on the under side of the cover glass a small grain of sugar, close to the water in which the spores of the fungi have produced filaments. The sugar will adhere to the moist surface of the glass. A thick ring of vaselin may be put on the edge of the glass ring cell, which prevents the cover glass from coming directly in contact with the glass cell and admits of a fine platinum wire being introduced between them without admitting air. When the streaming has ceased for any reason or become retarded, the particles of sugar may be shoved into the liquid containing the fungal filaments by means of the platinum wire and its effect observed immediately through the microscope. We have in this arrangement not such a change as might bring about streaming by the drier air of the room entering the glass cell when the filaments are growing. Streaming would, as shown before, occur by transpiration, if in the experiment here mentioned the filaments protruded beyond the edge of the drop. In such a case it would be difficult to determine how much of the accelerated or induced streaming is due to transpiration and how much to osmosis. In the method here outlined, however, the factor of transpiration is excluded and osmosis alone is responsible for the result. All the experiments tried in this way resulted, as expected, in streaming being induced or accelerated, and they thus confirmed the previous and similar ones of this paper. In like manner the result was confirmatory of and useful in the experiments just performed with ether. Where the streaming had been stopped by the addition of ether in any of the stronger solutions mentioned, it could always be awakened and caused to continue from 1 to 15 minutes, according to the strength of the ether, its time of action, and the quantity of sugar. The sugar was placed on the cover glass at the same time the ether was added to the specimen.

INJURY

Frequently injuries of various kinds, if not too severe, cause protoplasmic streaming in many plants.* Generally a rather serious injury is necessary to stop the protoplasmic movements permanently. Ternetz† found in *Ascophanus carneus* that by

* Pfeffer, W. Plant Physiology 2: 816–820 and literature there quoted. 1905.
† Ternetz, loc. cit. 282.

cutting an actively streaming part in two streaming ceased and did not recommence.

My experiments as regards injury have been performed on both *Mucor stolonifer* and *M. Mucedo*, and in both plants I obtained the same results. Schröter used *Mucor stolonifer* and *Phycomyces nitens*. I can confirm his results on *M. stolonifer*, but I was not successful in preserving *P. nitens* for this study.

When the filaments of actively growing specimens had their tips removed, a temporary increase in streaming was observed, due to the outflow of some of the contents and a relief of the pressure. This streaming could be traced back for a considerable distance among the filaments of the injured specimen. The streaming caused by such injury soon ceased. The specimens were then put away for 24 hours under favorable conditions and afterwards observed. They were found not to have been killed and were still in a living and streaming condition.

By separating the filaments of the fungi into two parts the specimens were not killed. They were observed after 18 hours and were found, Schröter states,* to have healed the wound. All of them were living, and streaming was either directly observable or was produced by the addition of a sugar solution.

A light pressure on the cover glass is sufficient to stop streaming temporarily and this, as Schröter states, will recommence when the pressure is relieved. The time of recovery of the streaming will depend on the amount of injury imparted and varies from an immediate recommencement to one delayed a few seconds. Pressure is, however carefully applied, too severe to excite streaming in these fungi or to accelerate that which may be present. A momentary acceleration may seem in some cases to be produced, but this is simply due to a partial compression of the filaments. When thisis relieved the streaming will continue as before.

INFLUENCE OF LIGHT

It has been known for a long time, as mentioned by Pfeffer,† that light may cause streaming. Also Ewart‡ makes reference

* Schröter, loc. cit. 17.

† Pfeffer, W. Plant physiology **3**: 318. 1905.

‡ Ewart, loc. cit. 71.

to this same point. Schröter has shown that light may cause or accelerate streaming, and I can confirm his observations on *Mucor stolonifer*, which he used, and also on *M. Mucedo*. These remarks refer to light of moderate intensity, for the light may be too strong for streaming to occur.

My experiments were carried out partly in daylight and darkness alternated and partly in gas light. In the first case the investigations were made by means of the freezing-apparatus of Molisch. With this arrangement the specimens could easily be kept at the desired temperature by means of warm or cold water siphoned in and out and the observation be carried on directly in the laboratory under the influence of light or dark. On heating the apparatus to the optimum temperature the specimens were kept first in the dark for two hours, when the protoplasm ceased to move. On admitting light a slow streaming began in five minutes and after a time became as rapid as the streaming of the control specimen. This experiment was repeated several times with the same specimen and always with the same result. When the temperature of the specimens was lowered from the optimum to 17° C. and then to 14° C., and the experiment repeated otherwise the same, the streaming became slower and slower on each new admission of light. Also, when the temperature was raised above the optimum but slightly (28° C.), the streaming on admitting light showed a rapid decrease in velocity. Too great heat or too intense light can readily, especially the former, make the protoplasm non-responsive. If, however, these are not continued too long the protoplasm of these fungi will recover in from one-fourth to one hour and stream as actively as before.

In the experiments made with the gas light an ordinary Welsbach burner was used and the investigations carried out in daylight and darkness repeated. The same results were obtained. In both of these series of experiments the specimens were preserved for 24 hours to see if in all cases they would become normal as compared to the controls for activity of streaming. At the end of this time both the specimens experimented on and the controls were normal and equally active.

TEMPERATURE

It has been shown by Hofmeister* and others long ago that a sudden change in temperature may excite streaming. More recently these observations have been confirmed by Hauptfleisch,† Ewart,‡ and others, for various plants. The same is referred to by Pfeffer,§ and Hörmann‖ observed the same result. The literature on this subject as regards the higher plants is too voluminous to enter into fully here, so that the above brief references must suffice.

As regards the fungi I can confirm the statements of Schröter¶ that temperature also has much to do with the ability of *Mucor stolonifer* to show streaming. *Phycomyces nitens* I have not studied in this respect. I have also found, as in the higher plants and as Schröter did for *Mucor stolonifer*, that a sudden change of temperature may produce streaming in those specimens of this fungus that were in a condition for such movement. The same I have found is true of *M. Mucedo*. This is the case whether the temperature is raised or lowered suddenly, but with the precaution that the change be not too great or continued too long.

I can not, however, agree altogether with Schröter** that the optimum temperature for streaming in *Mucor stolonifer* is 26° C. and for *Phycomyces nitens* 28° C. My studies have shown that the optimum temperature for streaming in *M. stolonifer* and *M. Mucedo* each ranges from 23° to 26° C. and for *P. nitens* from 26° to 29° C. These temperatures, as given by Schröter, seem to be too sharply drawn, for as stated I have found a difference of a few degrees Centigrade. I did not attempt to ascertain what effect the age of the hyphae had on the optimum temperature for streaming, if any exists, as is probable. Ewart†† has pointed out for some of the higher plants that it is almost impossible to ascertain the required temperature exactly or within a degree, owing to various factors which enter into the problem and which are not

* Hofmeister, W. Lehre von der Pflanzenzelle 53, 54. 1867.

† Hauptfleisch, P. Jahrb. Wiss. Bot. 24: 210. 1892.

‡ Ewart, loc. cit. 66 and literature there quoted.

§ Pfeffer, W. Plant Physiology 3: 316 and literature there quoted. 1905.

‖ Hörmann, G. Studien über die Protoplasmaströmung bei den Characeen 44. 1898.

¶ Schröter, loc. cit. 56.

** Schröter, loc. cit. 15.

†† Ewart, loc. cit. 60.

controllable by the experimenter. Nor can I agree to Schröter's* idea that streaming can be induced only in branched hyphae. I have caused streaming to occur in the filaments of *Mucor stolonifer* and *M. Mucedo* when unbranched as in FIG. 6, 7, and 8, when they were put under the optimum conditions of temperature, etc. Also, as stated heretofore, I have induced streaming in such unbranched filaments by osmosis and transpiration.

The filaments of *M. stolonifer* are not always filled with granular protoplasm before branching, as Schröter seems to think. In those cases where this is not the case streaming may easily occur. I performed some of my experiments of this kind in room temperature varying from 16° to 19° C., where fairly rapid streaming occurred, but it was noticeably slower than at the optimum temperature.

As also mentioned above, a sudden change almost immediately caused streaming. When a specimen whose protoplasm was still, but in a condition to stream, was suddenly cooled from 23° to 16° C. streaming was induced, or if heated from 16° to 19° C. or from 23° to 26° C. streaming was generally produced.

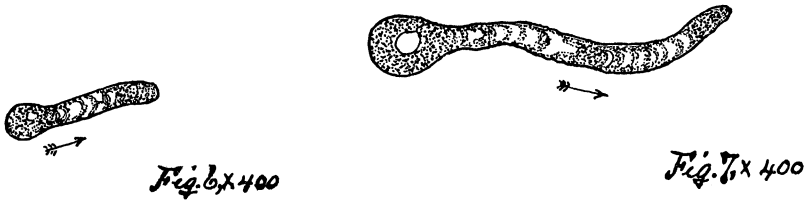
The movements of the protoplasm in the hyphae of several filamentous fungi have been described by Arthur,† Ternetz,‡ and Schröter.§ In *Mucor stolonifer* and *M. Mucedo*, both of which I have studied, the streaming shows no difference, so that my remarks on this point apply equally well to both. FIG. 9 shows a portion of one filament of *M. stolonifer* grown in a sugar solution. It was found that the rate of growth of these fungi, their size, and branching frequently varied greatly although grown under the same conditions. The streaming also showed a great difference as to velocity. Sometimes it was so slow as to be scarcely discernible, as for example when osmosis or transpiration was very feeble. On the other hand, streaming was fast or very rapid according as some factor such as those just mentioned was active. In those filaments that had attained some length numerous vacuoles were generally present, and these varied greatly in size

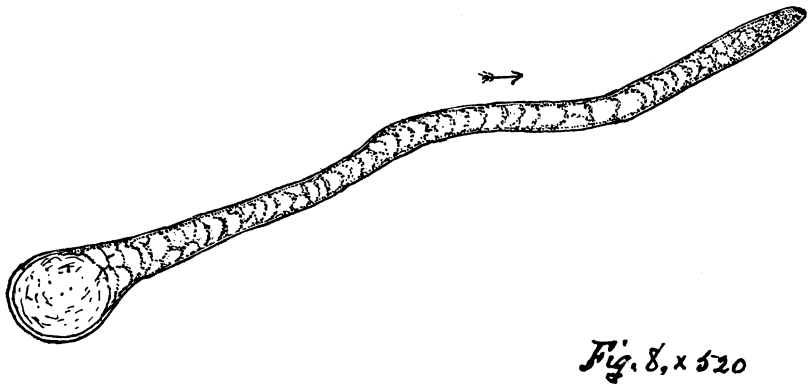
* Schröter, loc. cit. 15.

† Arthur, loc. cit. 493.

‡ Ternetz, loc. cit. 280.

§ Schröter, loc. cit. 4.





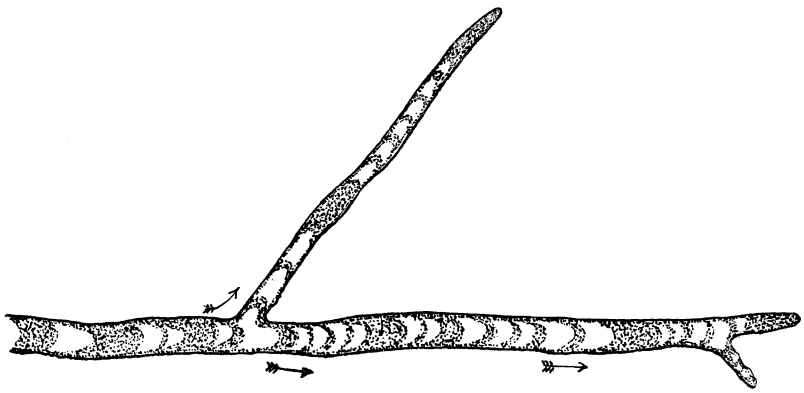


FIG. 6, 7, 8. Unbranched young filaments of *Mucor stolonifer*.

FIG. 9. Branched filament of *Mucor stolonifer* showing streaming and division of vacuoles at the point of branching.

The direction of streaming is in all cases shown by the arrows.

and shape, due mostly to the streaming back and forth. This caused some of the vacuoles to be separated into several or sometimes many smaller ones and in other cases caused small ones to fuse into one or more vacuoles.

The division of vacuoles, especially the larger ones, was well shown in those instances where a stream of protoplasm (FIG. 9) divided on coming to a branch. Then, frequently about one half or often only a small part would go into the branch. Whether the streaming is slow or fast the vacuoles are always carried along with the whole mass. They are convex on the end toward which the flow is directed and concave on the opposite end. This shows that a relief of pressure exists in the direction of streaming. Sometimes the streaming stops very suddenly with a jerk and when starting frequently does so in the same way, although the factors producing streaming are active. This kind of sudden cessation of motion generally lasts for from one to a few seconds, when streaming is resumed at the normal rate. It is caused, as careful observation will show, by a mass of temporarily impermeable protoplasm suddenly entirely plugging up the cavity of the filament at some point. As soon, however, as more water is removed, as for example by osmosis, transpiration, or some other factor, the pressure is gradually relieved and the obstruction suddenly gives way, allowing a recommencement of streaming so suddenly as to cause the protoplasm to appear as if jerked forward. Unless the obstruction is removed the filament beyond that point frequently collapses, due to excessive transpiration or other factor. Sometimes the velocity would vary for no apparent reason, as is known to be the case in other plants.

At times when no streaming was visible an extremely thin layer of protoplasm between the large vacuoles and the ectoplasm could be seen to be in motion. Part of the time it moved in the same direction as the streaming had moved and sometimes in the reverse direction. I was unable to see it moving in the opposite direction to the streaming protoplasm. I can therefore not agree with Schröter* on this point but find, as stated by Ternetz for *Ascophanus carneus*, that during streaming all of the moving protoplasm of *Mucor stolonifer* and *M. Mucedo* goes only in one direction.

* Schröter, loc. cit. 30.

It is hardly possible, even if a reverse movement did take place along the wall during the streaming, that it would be sufficiently active to account for the return of the protoplasm. The streaming occurs first in one direction, and when the factor that has caused this subsides or is overcome, it streams back in the opposite direction.

Streaming may be easily induced in *Mucor* and be caused to continue in first one direction and then the other without apparent harm to the plant for an indefinite time. This and other facts tend to show that it is not a "pathogenic" state of affairs as Keller* seems to think. The streaming may be of use in the long cells of these fungal filaments, as de Vries† suggests, to transfer substances. This, however, would not hold true for small cells as Ewart‡ has shown, for in such cases diffusion would distribute substances more rapidly than streaming.

SUMMARY

The foregoing experiments have proved the following points, most of which confirm Schröter's work, but some show his work in a few places to be incorrect:

1. The kind of nutrient media is of great importance for the proper growth of these fungi.

2. Streaming is caused in many cases by transpiration, and streaming is strong or weak according to the intensity of the transpiration.

3. Streaming is also caused in many cases by osmosis, as by the use of sugar. The streaming is always to the sugar. The rapidity of streaming depends on the concentration of the sugar solution.

4. During streaming caused by osmosis there is no peripheral streaming or movement in the opposite direction, as stated and figured by Schröter. This also confirms the statement of Ternetz.

5. Injury, as stated by Schröter, does not produce or accelerate

* Keller, J., cited from Pfeffer, loc. cit. 2: 818. 1905.

† De Vries, Bot. Zeit. **45**: 1. 1885.

‡ Ewart, A. J. On the ascent of sap in trees. Phil. Trans. Royal Soc. 40. 1905, quoted from Pfeffer, Phys. Pl. (Eng. Transl.) 3: 359. 1905.

streaming, but has a tendency to decrease any streaming that may be present. When the filament is cut into two pieces, only an outflow occurs. This may, in time, heal and streaming be resumed.

6. Light may cause and accelerate streaming when alternated with darkness in those fungal filaments that are in a condition for streaming.

7. A sudden change of temperature of several degrees will cause streaming in these fungi.

8. Contrary to Schröter's opinion, streaming may occur or be caused in unbranched as well as branched filaments.

My thanks are due Professor W. Pfeffer for placing at my disposal the facilities of his laboratory for this investigation and also for his constant interest and kind assistance.

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